NATIONAL RESEARCH COUNCIL
The Safety of Mono-and Diglycerides
for Use as Intentional Additives
in Foods

The Safety of Mono- and Diglycerides *for Use as* Intentional Additives in Foods

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Preface

In 1951, the Food Protection Committee formulated basic principles for the evaluation of the safety of food additives. In the light of these principles it has reviewed the available evidence concerning the safety of certain emulsifying agents in processed foods. A detailed study of this particular group of additives was undertaken because of the unresolved controversy resulting from the FDA Hearings to establish standards for bread and ice cream and because of the need for testing the usefulness of these general principles when applied to a specific problem. In a statement of November 9, 1951, the Committee reported that the available data were insufficient to permit a final judgment as to the safety of emulsifiers. The present report is a summary of the pertinent facts including those which have since been established concerning the mono- and diglycerides, together with an evaluation of the significance of these facts with respect to the question of safety. It is expected that later reports will be issued concerning the other types of emulsifiers.

Contents

I. Introduction

Monoglycerides and diglycerides have been well known as discrete chemical substances in the laboratories of organic and biochemists for nearly a century (1). Likewise, mixtures of mono- and diglycerides have been produced industrially for many years by reacting food fats (mainly triglycerides) with glycerine (2).

Utilization of these materials as food additives began during the period between 1928 and 1932. Addition of small amounts (one or two parts per hundred) of the Commercially produced materials then available, consisting of a mixture of mono-, di-, and triglycerides, achieved a modification of the functional properties* of both margarine and shortening. While exact dates of the original use of these materials both in the United States and in Europe have not been established, there are clear indications that by about 1927 the variability in certain functional properties of food fats used in the manufacture of margarine and shortening was thought to be due to variations in the mono- and diglycerides present in the starting materials and that the extent of their occurrence was dependent on such factors as ripeness of the oil seed and climatic conditions during its harvest and storage.

The development of shortenings containing mono- and diglycerides** during the last 20 years has continued to the point that today in the United States nearly every major brand of retail shortening carries about 2 per cent of added monoglycerides and 2 per cent of added diglycerides. Shortenings used by bakers for cake making carry a little over 3 per cent of each, and some shortening products designed for use in baking bread contain from 7 to 10 per cent of added monoglycerides and a like amount of added diglycerides. Mono- and diglycerides are used in margarine at a level somewhat lower than the maximum of 0.5 per cent allowed by the Federal Standard of Identity for Oleomargarine (3).

Other foods in which mono-and diglycerides are used in significant quantities as intentional additives are prepared baking mixes, ice cream, and confections. This list is not exhaustive, but still other foods containing these substances as intentional additives contribute to only an insignificant proportion of the total mono- and diglycerides entering the food supply.

Earlier considerations of the safety for long continued use of mono- and diglycerides were based on 1) indirect evidence of the normal occurrence of such materials in food fats and oils, and 2) the close relationship in chemical structure, comparable metabolism, and similar nutritive value, as compared with the triglycerides. These considerations have led to several decisions of governmental regulatory bodies concerning the use of mono- and diglycerides in foods, beginning with the definition in 1936 (4) of Edible Fats and Edible Oils as ". . . such glycerides of the fatty acids as are recognized to be wholesome foods." In addition to the action promulgating a Standard of Identity for Oleomargarine (1941)(5), there have been these actions providing for the op-

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* The term "functional properties" refers to characteristics which confer some advantage in use or impart certain desired qualities to a product, for example, emulsifying properties of shortening in cake, softening or tenderizing properties of shortening in bread, anti-weeping properties of margarine, etc.

** In this statement, the term "mono- and diglycerides" refers to partial glycerides of the fatty acids of recognized food fats, for example, of lard, of edible tallow, of hydrogenated vegetable oils, of coconut oil, etc. as they have appeared on the United States market for the last 40 years.

tional use of mono- and diglycerides in foods: 1) USDA, Meat Inspection Division (MID) accepted their use in margarine produced in part using meat fats (1936)(6); 2) FDA Suggested Ice Cream Standards (1947)(7); 3) FDA Tentative Bread Standards (1943) (8); 4) Standards of Identity for Cacao Products (1944) (9); 5) USDA, MID, approval for their use in shortening containing meat fats (1948) (10); 6) FDA Standards of Identity for Bakery Products (1952)(11).

II. Occurrence of Mono- and Diglycerides

A. In Food Fats and Oils

The scientific literature contains numerous references to the occurence of mono- and diglycerides along with triglycerides in food fats and oils (12, 13, 14, 15, 16, 17, 18, 19, 20). By analytical procedures proven usually with known pure compounds, many fats and oils were studied to determine their content of mono- and diglycerides. However, the available results were not conclusive in proving the occurrence of mono- and diglycerides because of the shortcomings of the methods.

More recently, any doubt as to the occurrence of mono- and diglycerides in food fats and oils has been removed by the isolation and positive identification of monoglyceride from hog pancreas tissue (21, 22), of monoglycerides from lard and bread (23), and of monoglyceride from beef fat (22). Moreover, periodic acid analyses on surface-active concentrates obtained by partition chromatography from rice bran oil, soybean sprouts, white flour, cracked wheat flour, whole wheat flour, potato chips, and beef pancreas indicate the presence of monoglycerides (22). In most cases, the amounts of monoglycerides have been found to occur in the order of one-half to one per cent of the fat contained in the food.

B. *Formation of Monoglycerides During Food Preparation*

A partial hydrolysis of triglycerides to monoglycerides has been observed to take place during baking of bread and cake. Quantitive data are fragmentary but serve as an indication at least of the order of magnitude. In one intensive study the lipids were extracted from several types of bread and the monoglycerides isolated. They were identified by periodic acid oxidation, fatty acid analysis, glycerol recovery, counter current extraction, and infra-red absorption (23). The lipid extracted from bread contained from 10 to 20 per cent monoglycerides although no monoglycerides had been employed in the shortening.

Unpublished results (Communication from the Distillation Products Industries, Rochester, N. Y.) show the transformation of 13.7 per cent of the shortening to monoglycerides during the baking of cake.

Similar observations have not been reported for the transformation of other food fats into monoglycerides during cooking, nor has the extent of diglyceride formation been reported.

C. *Formation During Digestion Of Triglycerides*

Studies on the digestion of triglycerides have provided indirect evidence that mono- and diglycerides are produced during the process. Frazer's observations (24, 25, 27, 28, 29, 30, 31, 32, 33, 34) led him to conclude that monoglycerides are formed in the small intestine as the result of partial enzymatic hydrolysis of food fat triglycerides. Likewise, Reiser *et al* (26) concluded that the major portion (55 to 75%) of ingested fat was hydrolyzed and absorbed as monoglycerides. *In vitro* studies by numerous investigators (25, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47) provided additional support for this concept.

The recent development of several new methods of separating and analyzing mixtures containing mono-, di-, and triglycerides enabled other workers to demonstrate the appearance of mono- and diglycerides in the intestinal tract of experimental animals and man as products of digestion of

food fats. Kuhrt *et al* (47) have recently reported a study on fat digestion in man in which hydrolysis of ingested fat containing only 0.15 per cent monoglycerides resulted in the appearance of a substantial amount of monoglycerides in the upper section of the intestine within one to two hours after ingestion of a test meal. From one-third to one-half of the total lipid in the upper intestinal tract was isolated as purified monoglycerides which had the same characteristics (shown by periodic acid titration, fatty acid analyses, glycerol recovery, counter current extraction, and infra-red absorption spectra) as those prepared in the laboratory by super-glycerinating milk fat which was the predominant fat of the ingested food.

The experimental studies of fat digestion by Mattson, Benedict, Martin, and Beck (48) confirm and extend the results of Reiser *et al* and Kuhrt *et al* on the appearance of large amounts of monoglycerides in the intestine following the ingestion of a meal containing triglycerides. Mattson *et al* adapted a combination of methods (periodic acid reaction, hydroxyl value, counter current distribution analysis, X-ray analysis and infra-red absorption spectra) to the quantitive estimation of both monoglycerides and diglycerides in the same samples of lipids isolated from the intestinal tract. Using the total lipid extracted from the whole small intestine of rats following the feeding of triglycerides, these authors found that the mono- and diglyceride content was 16 and 36 per cent, respectively. They also report the finding that the monoglycerides present were mixtures of 1- and 2-monoglycerides. The ratio of 2-monoglyceride to 1-monoglyceride varies from approximately 1:3 to 2:3 in their experiments. There was also evidence that the 2-monoglyceride was formed as the predominant initial form (as much as 90 per cent of the total monoglycerides) and that isomerization to 1-monoglyceride occurred in the intestine.

III. Amounts of Mono- and Diglycerides Intentionally Added to Foods

Based on food consumption surveys, it is clear that a relatively small group of processed foods account for more than 90 per cent of all processed foods consumed. Knowledge of the intentional additives in this selected list provides, therefore, a reasonably accurate estimate of the quantity of additives in the diet. The considerations which follow are based upon a survey of the home food consumption in urban families made in the spring of 1948 by the Bureau of Human Nutrition and Home Economics (50). The data in this survey are in essential agreement with the results of other surveys by the Bureau of Agricultural Economics (49) and by the Committee on Fats of the Food and Nutrition Board, National Research Council (51).

Some 304 individual foods and groups of foods are reported in the survey of home consumption by urban families. For many of these foods there is no problem of intentional additives. They are either sold in the fresh form or processed in such a simple manner that their composition is essentially unaltered. These items include such fresh foods as fruits, vegetables, potatoes, meats, and eggs. Whole milk can be included among the fresh or unaltered foods since the pasteurization process does not involve an important change in composition. Although milk may contain minute amounts of emulsifier serving as carrier for vitamin D addition, those quantities are considered too small to contribute significantly to the total of emulsifiers in the diet. The remaining foods are those which are important to consider from the standpoint of chemical additives. According to the survey, the consumption of processed foods amounts to 43 per cent of the total diet. The relative importance in the diet of the major groups of processed foods is given in Table I. This table lists 73 individual food items which in total constitute 93 per cent of the consumption of processed foods reported in the survey.

For those foods which are covered by specific government regulations it is possible to make estimates of the amounts of additives which would be included in the diet if every processor used the maximum allowable quantities. Forty-one of the selected processed foods in Table I are foods whose composition is specifically controlled by federal laws and regulations or which have been the subject of hearings on proposed standards of identity. In the case of those 41 foods, there is available considerable information on the additives which are permitted.

Mono- and diglycerides have been reported to be used in bread, cakes, cereal mixes, ice cream, margarine, shortening, lard, milk drinks, whipping cream substitutes, candy, and peanut butter. In estimating the maximum forseeable consumption of mono- and diglycerides, certain of the above uses can be neglected because of the small quantities involved. Doughnuts, cereal mixes, milk drinks, and whipping cream substitutes do not appear in the selected list of major processed foods. Of all the foods listed in Table I, there are nine in which mono- and diglycerides are known or reported to be used. These foods are shown in Table II together with the estimated maximum level of use of added mono- and diglycerides and the resulting figures for this maximum daily consumption. It is recognized that many brands of the foods listed in Table II may

have either no added mono- and diglycerides or less than the maximum permissible amount.

Bureau of Census data on the total production of shortening and the amount of glycerine used in the shortening make possible a direct calculation of the amount of mono- and diglycerides used in shortening and the per capita consumption of mono- and diglycerides from this source. As might be expected, the quantity actually used is smaller than the estimated maximum amount which might be used. The direct figure, however, serves as a reasonable check on the accuracy of the estimated maximum. During 1950 the total shortening produced was 1,709,907,000 pounds. The amount of glycerine used in shortening was 4,446,000 pounds. Since it takes 8.3 pounds of glycerine to make 100 pounds of mono- and diglyceride concentrate, there were 53,566,000 pounds of concentrate used in the shortening. This means that all shortening produced contained an average of 3.1 per cent mono- and diglyceride concentrate. The per capita consumption can be calculated to be about 0.4 grams per person per day (67).

IV. Nutritive Value of Mono- and Diglycerides

Many studies with experimental animals have established the fact that no significant differences in nutritive value are observable when either purified monoglycerides or mixtures of mono- and diglycerides ("superglycerinated edible fats") have been used in the experimental diet in place of the triglycerides. Criteria employed in these experiments have included measurements of rates of body weight increases, food consumption data, reproduction through several generations, lactation, digestibility and absorption, deposition of fatty acids in animal tissues, possible influence on absorption or metabolism of other substances, and gross and microscopic examination of tissues for any evidence of deviation from normal tissues (52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65).

V. Surface Activity of Mono- and Diglycerides

Among the important physical properties of the mono- and diglycerides, and one that contributes to the effectiveness of their use as additives in foods, is the emulsifying property which, in turn, is a function of the surface activity of the materials. Until recently, no systematic studies had been made of the boundary tension relationships in systems resembling those appearing during the digestion of fats. Particularly, data were lacking for the effects of mono- and diglycerides, bile salts, or fatty acids on oil-water boundary tensions in such systems.

Studies of such systems (66) indicate that in monoglyceride-water-triglyceride systems, maximum effects (minimum interfacial tensions) are produced at concentrations of one to two per cent of monoglycerides, and added quantities have no further effect. When bile salts and oleic acid are added to such a system (simulating more closely the conditions in the intestine) maximum effects are produced at monoglyceride levels as low as one-half per cent, and higher levels of monoglycerides produce no further effect.

A system simulating that occuring in the intestine during fat digestion is characterized by a very low oil-water boundary tension as a result of the quantities of natural emulsifiers, bile salts, and fatty acids, normally present. The low boundary tensions are observed even without monoglycerides, also normally present. In fact, boundary tensions are so low that large quantities of monoglyceride could be added without significantly reducing the surface tension in such systems.

When compared with other naturally occuring emulsifiers (e.g. mixed bile salts, sodium cholate, sodium glycocholate), monoglycerides are the least surface active. Bile salts complexed with fatty acids would be expected to show even greater surface activity at an oil-water boundary than bile salts alone.

VI. Evaluation

The available information on the natural occurence of mono- and diglycerides in food fats, their formation during cooking and digestion, and the amount intentionally added to foods allows an approximate estimation of the maximum quantities likely to be present in the diet from all sources. Although the data are admittedly fragmentary, the quantitive estimation can be based on reasonable assumptions and is valid at least with respect to the order of magnitude.

On the basis of present information, it is clear that monoglycerides occur in food fats at least to the extent of 0.5 per cent and that diglycerides are present in approximately the same proportion. As the per capita consumption of fat is approximately 100 grams per day, the resultant per capita consumption of mono- and diglycerides is approximately one gram per day.

Additional monoglycerides may also be expected to be present in the usual human diet as a result of their formation during baking and cooking of processed foods. It is estimated that an additional one to two grams per person per day may be consumed.

Most important to these considerations is the appearance of substantial quantities of mono- and diglycerides in the intestine during digestion. To date no exact data for fat digestion in man are available to indicate the extent to which the total ingested fat may be transformed to mono- and diglycerides. In studies with experimental animals, more than one-half of the ingested fat can be accounted for as mono- and diglycerides in the intestine. It is reasonable at this time to assume that as much as 30 to 50 grams of mono- and diglycerides per day may be formed during digestion of 100 grams of ingested fat.

In contrast with these amounts of mono- and diglycerides that are naturally occurring in foods or in the intestine during digestion of fats, it appears that the maximum amount of mono- and diglycerides intentionally added to all foods cannot be expected to exceed approximately two grams per person per day. This positive demonstration of relatively large amounts of naturally-occurring mono- and diglycerides obviates the necessity for an extensive program of toxicity testing such as is recommended in this Committee's statement of Basic Principles (68). The additional amounts which may be intentionally added to foods are not large enough to raise the consumption to levels significantly higher than normal.

The question has been raised as to whether or not the property of altering interfacial tension may alter important physiological processes. Evidence on the surface activity of mono- and diglycerides demonstrates that the additional amounts provided by their use as intentional additives in foods have no significant effect in altering interfacial tension in the intestines in the present of the relatively large amounts of these and other natural surface active materials.

The conclusions in this report apply to mono- and diglycerides of the fatty acids of recognized food fats and do not generally apply to materials such as monolaurin and sulfo-acetate derivatives of mono- and diglycerides.

The mono- and diglycerides are normal nutrients, equivalent to triglycerides and giving rise to identical products in digestion and metabolism. When the available

facts are examined in the light of the statement of Basic Principles Involved in Evaluating Safety in the Use of Chemical Additives in Foods (68), there is no evidence on which to question the safety of mono- and diglycerides as food additives.

Table I. *Consumption of Major Processed Foods*

	Pounds eaten per household[*] per week	
	Major groups	*Major individual foods*
Manufactured dairy products excluding butter	4.612	
Cream		.487
Evaporated milk		1.550
Ice cream		.805
American cheese		.484
Cottage cheese		.340
Buttermilk		.800
Fats, including butter	2.953	
Butter		.759
Margarine		.601
Lard		.383
Shortening		.450
Salad oil, cooking oil		.205
Mayonnaise, French dressing		.295
Salad dressing		.260
Flour	2.832	
Plain white flour		1.718
Self-rising flour		.305
Prepared flour mix		.316
White whole ground corn meal		.482
Cereals and Pastes	1.728	
Rice		.324
Rolled oats, oatmeal		.217
Ready-to-eat cereals		.465
Macaroni, noodles, and spaghetti		.477
Bread	6.139	
White		4.900
Whole wheat		.556
Cracked wheat, rye, raisin		.683
Baked goods other than bread	2.063	
Rolls, biscuits, muffins		.274
Crackers		.402
Cake		.472
Processed meats	2.303	
Uncooked smoked ham		.357
Bacon		.701
Bologna, frankfurters, meat spreads, potted meats, spiced ham, Vienna sausage		.951
Sugar and sweets	4.105	
White sugar		2.782
Corn syrup		.141
Maple syrup		.176
Jellies, jams		.376
Candy		.364
Dried and frozen fruits and vegetables	1.308	
Dried fruits		.231
Dried beans		.371
Frozen fruit		.303
Frozen vegetables		.221
Canned fruits and vegetables including juices	7.146	
Canned fruit		1.708
Baked beans		.288
Snap beans		.259
Corn		.446
Peas		.634
Tomato pulp		.478
Grapefruit juice		.665
Orange juice		.715
Tomato juice		.612
Beverages	6.632	
Beer		1.911
Ground bean coffee		1.047
Bottled soft drinks		3.322
Miscellaneous	1.983	
Catsup, chili sauce		.246
Pickles, olives		.371
Soups		.797
Prepared dishes		.363
Peanut butter		.206
Total	43.804	40.052

[*] In this study, a household is defined as 3.42 persons.

TABLE II. *Estimate of Maximum Foreseeable Per Capita Consumption of Added Mono- and Diglycerides in Processed Foods*

	Average amounts eaten per household per week (3.42 persons)	*Average amounts eaten per person per day*	*Estimated maximum level of use of mono- and diglycerides*	*Maximum daily consumption of mono- and diglycerides*
	POUNDS	GRAMS	PER CENT	GRAMS PER PERSON
Bread	6.139	116.1	0.8*	0.93
Baked goods	1.148	21.7	0.5	0.11
Shortening	.450	8.5	6.0**	0.51
Lard	.383	7.2	6.0	0.43
Margarine	.601	11.4	0.5***	0.06
Prepared flour mix	.316	6.0	0.5	0.03
Ice cream	.805	15.2	0.2***	0.03
Confections	.364	6.9	0.1	0.01
Peanut butter	.206	3.9	0.5	0.02
				2.13

* Based on a figure of 4 pounds of shortening per 100 pounds of bread and a top value of 20% mono- and diglyceride in shortening sold to bakers.

** Based on information supplied by members of the food industry that shortening sold for home consumption may contain up to 6% of mono- and diglycerides.

*** Based on existing and proposed Standards of Identity.

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